

## **EXHIBIT D**

TITLE Specificity studies w/ Chlamydia probes

From Page No. 34

Prepare hybridization mixes  
lysates:

300 $\mu$ l DIBSS diluent  
10  $\mu$ l lysate  
360  $\mu$ l DIBSS / probe  
400  $\mu$ l

Incubate 1 hr 64°C

Do duplicates  
Pos Control

300  $\mu$ l DIBSS  
1  $\mu$ l Chlamydia RNA + 9  $\mu$ l H<sub>2</sub>O  
360  $\mu$ l DIBSS / probe  
400  $\mu$ l

Neg. control

300  $\mu$ l DIBSS  
10  $\mu$ l 3.2% SDS  
360  $\mu$ l DIBSS / probe  
400  $\mu$ l

Add 4.5 ml Separation solution .14M PB LN 60278

Incubate 5' at 64°C

Centrifuge

Decant SN into 35ml scintillation vial

Add 5.0 ml Wash solution, vortex LN 60276 .14M PB

Incubate 5' at 64°C

Centrifuge

Decant SN into same scintillation vial as 1st wash

1. Count HA pellets and wash fraction for <sup>32</sup>P counts

2. Add 5.0 ml cytosecint to HA pellet

Add (15.0 ml) p.gel to wash vial

Count again in jet <sup>145</sup>T counts

Used Sol for counting 2x2'

Prepare blanks ① spin down 4.5 ml HA and count

② add 9.0 ml .14M wash and to scint. vial and count

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Witnessed &amp; Understood by me,

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From Page No. 38

Purpose: To assay lysates of Chlamydia strains for cross reactivity with probes at 60°C and 176 at 64°C.

Reagents: Same as for previous experiment (405:34)

In addition used lysates for C. trachomatis 300, 301, 302, 303 (other serotypes than the one used to make the probes and found in humans)  
From Mary NB 435:3

Diluted HII RNA .25 ug./ul (334:10) 1:5 to .05 ug./ul

Made up probe solutions

① To 8.4 ml DIBSS mix, add 5 ul <sup>3</sup>H/176 ( $1.3 \times 10^6$  cpm/ml)  
NB-391:25 mixed and 20 ml of  
Probe 1082 (22,000 cpm/ml.) iodinated by  
Kevin NB 388:42

②

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TITLE Specificity Studies with Chlamydia probes

Pr j ct N . 3  
Bo k N . 405

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Assay Protocol: Prepare hybridization mixtures in scintillation vials.

Lysates: 30 µl DIBSS diluent  
10 µl Lysate  
360 µl DIBSS/probe mix  
400 µl total volume

N.C.: 30 µl DIBSS diluent  
10 µl 3.2% SDS  
360 µl DIBSS/probe mix  
400

711 RNA: 30 µl DIBSS diluent  
2 µl RNA 0.5 µg/ml  
8 µl H<sub>2</sub>O  
360 µl DIBSS/probe  
400

E.coli RNA: 30 µl DIBSS diluent  
10 µl RNA 10 µg/ml  
360 µl DIBSS/probe  
400

Incubate ChtA 176 probe rxn at 64°C 1 hr.

Separate hybrids and wash <sup>135</sup> 405 as p. 35

Count in HAL 2x2' on  
to get <sup>32</sup>P counts Program 1

Add 0.09 ml of probe mixture to HA pellet in scint vial  
Add 5.0 ml cytosinct and count use this value  
for total counts for <sup>135</sup>I-hybridization w/ pan bacterial prob  
Add 5.0 ml cytosinct, and count in HAL 2x2'  
to get <sup>135</sup>I counts. etc. Program 1 <sup>135</sup>I-7082  
(Did not count washes for <sup>135</sup>I as did in previous expt)  
E.coli RNA sample 2 was spilled for probe ChtA 176.

Results: see p. 41

Conclusions: Probes 176 and reacted with all C. trachomatis  
strains but not with the C. psittaci. The <sup>135</sup>I-7082  
data showed that RNA was present in all tubes

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TITLE Specificity Study w/ Chlamydia probesProj ct N . 3  
B k N . 405

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		Cat A 176 EcoA 182	340	
N	SMPL	CPM	TOTAL	%
711	1 FP	929.8	2091.0	44.5
	BP	1161.3		55.5
802	2 FP	942.3	2166.0	43.5
	BP	1223.8		56.5
301	3 FP	623.0	2210.0	28.2
	BP	1587.0		71.8
302	4 FP	631.8	2179.3	29.0
	BP	1547.5		71.0
303	5 FP	561.5	2180.3	25.8
	BP	1618.8		74.2
304	6 FP	536.8	2159.5	24.9
	BP	1622.8		75.1
794	7 FP	566.5	2147.5	26.4
	BP	1581.0		73.6
767	8 FP	610.8	2248.3	27.2
	BP	1637.5		72.8
768	9 FP	551.3	2178.0	25.3
	BP	1626.8		74.7
711	10 FP	563.5	2124.0	26.5
	BP	1560.5		73.5
794	23 FP	2278.5	2295.0	99.2
	BP	16.5		.7
767	12 FP	2313.5	2328.0	99.4
	BP	14.5		.6
768	13 FP	2228.5	2239.3	99.5
	BP	10.8		.5
768	14 FP	2284.0	2295.5	99.5
	BP	11.5		.5
711	15 FP	2303.3	2319.5	99.3
	BP	16.3		.7
711	16 FP	2302.5	2318.3	99.3
	BP	15.8		.7
711	17 PNA	403.0	2152.0	18.7
	BP	1749.0		81.3
711	18 FP	310.0	2073.8	14.9
	BP	1763.8		85.1
711	19 FP	2202.8	2217.3	99.3
	BP	14.5		.7
NC	20 FP	590.8	597.3	98.9
	BP	6.5		1.1
NC	21 FP	2290.5	2316.3	98.9
	BP	25.8		1.1
NC	22 FP	2255.3	2278.0	99.0
	BP	22.8		1.0

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TITLE Specificity Studies with Cta A

From Page No.

Purpose: Results:

## ECOR 1082 with 176

TOTAL 1: 13760

SAMPLE	CPM	HR%	AVG%	XBKGD
311	6849	49.8		
	7170	52.1	50.9	
300	8420	61.2		
	8571	62.3	61.7	
301	9490	69.0		
	9610	69.8	69.4	
302	8809	64.0		
	9557	69.5	66.7	
303	9153	66.5		
	8793	63.9	65.2	
754	7520	54.7		
	7666	55.7	55.2	
767	7692	55.9		
	7477	54.3	55.1	
768	4677	34.0		
	4483	32.6	33.3	
RNA 711	8877	64.5		
	9516	69.2	66.8	
cult.	8431	61.3	61.3	
N.C.	139	1.0	2.4	
	132	1.0	1.0	

## % Hybridization

Lysates	<sup>32</sup> P-CATA 176	<sup>125</sup> I-EcoA 1082
C. trachomatis 711	56.0	50.9
" 300	71.4	61.7
" 301	74.5	69.4
" 302	73.2	66.7
" 303	74.1	65.2
C. psittaci 754	0.7	55.2
" 767	0.5	55.1
" 768	0.7	33.3

## RNA

C. trachomatis 711	83.2	66.8
E. coli	0.9	61.3
N.C.	1.1	1.0

Hybridization  
Assays done at 60°C.

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